Peptides corresponding to M2TM wild-type (M2TM) and M2TM S31N (S31N_TM) domains were chemically synthesized. M2TM peptides were labeled at: d4-Ala_{29}/^{15}N-Ile_{42}, or d8-Val_{27}/^{15}N-Ala_{29}, or d2-Gly_{34}/^{15}N2-Trp_{41}, or d5-Trp_{41}/^{15}N-Ile_{39}. An S31N_TM peptide was labeled at d8-Val_{27}/^{15}N-Ala_{29}. ^{2}H and ^{15}N static NMR spectra of the peptides reconstituted in DOPC:DOPE bilayer at pH 7.5 were acquired.
Analysis of the spectra of M2TM(d4-Ala_{29}) at various temperature and echo times indicates that above the phase transition temperature of the lipids M2TM channel is undergoing rotation motion about the bilayer normal and channel axis on the ms time scale. The spectra of M2TM(d4-Ala_{29} and d8-Val_{27}) in the absence and presence of Amt indicate that drug binding doesn't affect spectral line shape and is proposed to have no significant influence on backbone and side chain dynamics of these sites. The S31N mutation lead to narrowing of the spectrum of S31N_TM(d8-Val_{27}) in comparison to M2TM(d8-Val_{27}). This mutation was reported to increase the splay between helices which could lead to a less tight packing of Val_{27} side chains and an increase of side chain dynamics. The collapsed ^{2}H powder patterns of M2TM(d2-Gly_{34} and d5-Trp_{41}) at 288 K indicates that both sites are undergoing motions on μs/ms time scale. Moreover, at 297 K the collapsed powder pattern of 15Nε-Trp_{41} indicates that the indole ring is undergoing large amplitude motions, possibly about $\chi1$ and $\chi2$.

1282-Pos Board B12
Rotation Triggers Nucleotide-Independent Conformational Transition of the Empty Beta Subunit of F1-ATPase
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F1-ATPase (F1) is the catalytic portion of ATP synthase, a rotary motor protein that couples the proton gradient to ATP synthesis. When driven by a proton flux, the F1 asymmetric gamma subunit undergoes a discrete rotation inside the catalytic headpiece and causes the binding sites located at the beta subunits to cycle between states of different affinity for nucleotides. These concerted transitions drive the synthesis of ATP from ADP and phosphate. Molecular details of the coupling between the mechanical progression of gamma and the conformational state of the catalytic headpiece are not fully understood.
Here, we study conformational dynamics of the nucleotide-free beta during the catalytic cycle. Using molecular dynamics simulations, we show that this subunit, initially in the open, low affinity state, undergoes a fast closing transition in response to the gamma rotation in the synthesis direction. By computing a free energy profile, we further find that the initial transition to the half-open state is driven by the intrinsic elasticity of beta, dominated by the electrostatic interactions between elements of the active site. Therefore, our data suggest that ADP binding to F1 occurs via conformational selection and is preceded by the transition of beta to the half-open state. Elastic properties of beta imply that gamma is unlikely to be pulled into the position seen in the x-ray structure by spontaneous opening of the empty beta. Instead, the observed position is stabilized by interactions with the two other beta subunits and keeps the empty beta in the fully open conformation.

1283-Pos Board B13
Interdomain Dynamics of Phosphoglycerate Kinase Studied by Single-Molecule FRET and a Mesoscale Hydrodynamics Simulation
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Well pronounced interdomain movements in 3-Phosphoglycerate kinase (PGK) are assumed to be crucial for the reversible phosphor transfer reaction catalyzed by this enzyme during glycolysis. Using a cysteine double mutant with fluorescent dyes attached at the distal ends of each domain of PGK from yeast [1], we performed single-molecule Förster Resonance Energy Transfer (smFRET) experiments. The fast dynamics of the protein were simulated with an elastic network (EN) under a Multiparticle Collision Dynamics (MPC) approach, combined with an accessible volume (AV) description of the dye [2]. 2D-plots of the FRET-efficiency versus the donor lifetime [3] show that PGK is a highly flexible system with interdomain dynamics spanning from nanoseconds up to milliseconds. Here, slow interconversion between an extended state and a compact conformation of the domains take place. The internal dynamics of the compact state is faster than milliseconds. Starting from the compact state, hinge bending brownian fluctuations bring the ligand-free protein in the catalytically competent state. The character of this functional motion is encoded in the structural topology of PGK as shown by normal mode analysis (NMA). Upon addition of the substrates the expanded state depopulates, with a population shift mechanism selecting the compact conformation which better allows the functional relevant motions. The timescale of the interdomain motions is recovered by means of the mesoscale hydrodynamics simulation.

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1284-Pos Board B14
Determinants of Fibrinolysis in Single Fibrin Fibers
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To prevent local hemorrhage upon injury, a blood vessel will constrict to prevent blood loss; platelets will aggregate at the injury site and trigger the coagulation cascade, forming a platelet plug held together by fibrin fibers. When the hemostatic role of a thrombus is fulfilled, it is effectively removed from the circulatory system via the fibrinolytic system, which is mediated by the serine protease plasmin. Throughout the fibrinolytic process, fibrin fibers undergo significant morphological and biochemical changes as thrombi are digested by plasmin. Understanding the digestion of whole blood clots requires fundamental knowledge of how various structural and physical properties affect the rate of fibrinolysis at the level of single fibrin fibers. We have identified and characterized a novel pathway in plasmin-induced fibrin degradation where fibrin fibers remain intact and exhibit an increase in length and Brownian motion during fibrinolysis. Other members of our lab have identified the αC-domains as the dominant source of low strain (<100%) fiber extension and relaxation. Since elongated fibers exhibit a strain of <20%, our results indicate that elongation occurs when plasmin predominately cleaves the αC-domains of fibrin molecules, causing the fiber to relax. These results also suggest a fiber's initial diameter and prestrain determine whether a fiber will lyse or elongate.

1285-Pos Board B15
Internal Motions Prime cIAP1 for Rapid Activation
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Cellular inhibitor of apoptosis 1 (cIAP1) is an E3 ubiquitin ligase with roles in both apoptosis and NF-κB signaling. Recent crystallographic and SAXS data have shown that the ligase activity of cIAP1 is autoinhibited by occlusion of the RING dimerization interface through several inter-domain interactions, most critically in the BIR3:RING interface. This interaction is disrupted by SMAC and SMAC mimetics, initiating cIAP1 dimerization through its newly exposed RING domain and activation of its E3 ligase activity. Activation of cIAP1's ligase activity leads to cIAP1 autoubiquitination and proteasomal degradation. Intriguingly, we observed that although the binding site for cIAP1's E2 partner enzyme is occluded in the crystal structure, E2 binds to both the monomeric and dimeric forms of cIAP1 with nearly the same affinity. This unexpected result indicated that there were likely significant residual motions occurring within monomeric cIAP1. Through NMR relaxation dispersion measurements and resonance broadening we have been able to detect significant motions occurring on relatively fast, microsecond-millisecond timescales at specific domain interfaces present in the autoinhibited form of cIAP1. In addition, time-resolved SAXS measurements conducted in line with a stopped flow injection system have allowed us to measure the opening rate of cIAP1, which occurs over hundreds of milliseconds. The tight regulation of apoptosis is essential in multicellular organisms and the proteins involved in transducing pro-apoptotic and anti-apoptotic signals are no doubt under stringent evolutionary control. The internal motions present in cIAP1 likely arose from the competing pressures to activate apoptosis as quickly as possible while maintaining the fidelity of the pro-apoptotic signal.

1286-Pos Board B16
The Free Energy Contribution of SH3 and SH2 in c-Abl 1b Autoinhibition Mechanism via a Computational Structure-Based Model
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We report on a computational analysis of the dynamical and structural arrangements induced by the presence of the regulatory domains SH2 and SH3 in the simulated construct of protein c-Abl 1b. Our approach is based on a hybrid structure-based all-atom model combined with the parallel tempering metadynamics method to enhance conformational sampling. The combined benefits of these two approaches allow the study of events whose time scale is well beyond the capabilities of standard all-atom molecular dynamics simulations. This